Amendments to the Specification

Please amend paragraph [0001] at page 1 of the specification as follows:

[0001] This Application is the U.S. National Phase of International Application PCT/US2004/013031, filed April 29, 2004, which is a continuation-in-part of Application No. 10/425,037, filed April 29, 2003.

[0002] This application entirely incorporates by reference herein ~~is a continuation-in-part of~~ PCT/US02/34419, filed October 29, 2002, ~~which designates the United States and will be published in English; and that claims benefit of priority of~~ and U.S. Application No. 09/984,664, filed October 30, 2001~~; both Applications being incorporated by reference herein~~.

Please amend paragraph [00132] at page 48 as follows:

[00132] FIG. 11: Deamination conversion of unmethylated cytosine groups in DNA. Deamination converts unmethylated C to U. Methylated C groups, such as those in CpG islands that regulate eukaryotic genes, are resistant to deamination and remain as C in the product DNA. If 100% deamination occurs, methylated DNA will still contain CpG doublets, whereas unmethylated DNA will contain no cytosine and will now contain UpG where CpG doublets were before deamination. This difference in DNA sequence can be used to distinguish between methylated and unmethylated DNA by transcription because the two DNAs encode different dinucleotides (SEQ ID NOs: 2, 3, 4 and 5).

Please amend paragraphs [00135] and [00136] at pages 49-50 as follows:

[00135] Similarly, abortive synthesis of trinucleotides by transcription initiation with labeled dinucleotides that end in C (ApC, CpC, GpC, UpC) and termination with labeled GTP can be used to produce signal from the deaminated methylated template, but not the deaminated unmethylated template. This trinucleotide synthesis approach may be expanded by the addition of a site-specific oligonucleotide to allow

assessment of the methylation status of a specific CpG site, rather than the entire island, as illustrated in Figure 13 (SEQ ID NOs: 6 and 7).

[00136] FIG. 13: Assessing methylation status of specific CpG sites in CpG islands by abortive initiation. Target site probes can be used to examine the methylation status of specific CpG islands in specific genes. In the deaminated methylated DNA, the dinucleotide CpG is encoded by the template at the 3 methylated sites 1, 3 and 4, but not by the unmethylated site 2. To specifically determine if Site 3 is methylated and if so, to what extent, position (C21) can be targeted with a Target Site Probe, as described in Figure 9. The template C in question is positioned at the junction of the bubble region and the downstream duplex so that it encodes the next incorporated nucleotide for appropriately primed RNA polymerase that binds to the bubble region. If a labeled initiator R_1-N_XpC-OH is used, where N_X may be C for a dinucleotide CpC initiator or N_X may be CpC for a trinucleotide initiator, etc., the initiator can be elongated with a labeled GTP analog pppG-R_{2G} to form a trinucleotide R_1N_XCpG-R_2. Similarly, if the C in question was not methylated, the position will now be a U and will encode nucleotide A. If an ATP analog pppA-R_{2A} is present, it will be incorporated opposite positions where the C was not methylated. If the GTP analog is labeled with group R_{2G}, which is an energy acceptor from the R group on the initiator, R_1, then the amount of $R_1N_xCpGR_{2G}$, which will be proportional to the amount of methylated C present at that position, can be quantified by measuring the emission from R_{2G} at wavelength λ_{2GE}. The similar situation exists for incorporation of the ATP analog and measurement of the emission from its R group, also an energy acceptor from the initiator R_1. By determining the ratio of the magnitude of emission from the GTP analog to the total emission from both the ATP and GTP analogs, the site can be assigned a methylation index M. If all of the Cs at that position are methylated, M = 1. If none of the site is methylated, M = 0 (SEQ ID NOs: 8, 9 and 10).

Please amend paragraph [00140] at pages 51-52 as follows:

[00140] FIG. 17: Signal Generation from artificial promoter. An Artificial Promoter Cassette (APC) consists of one or more oligonucleotides or polynucleotides that together create a specific binding site for an RNA polymerase coupled to a linker region (APC linker) for attachment to target molecules (DNA, RNA, Protein). The APC may contain an artificial promoter, or it may contain the promoter for a specific RNA polymerase. For example, trinucleotide or tetranucleotide products that could be generated from with a common phage RNA polymerase can be made with a labeled GpA or GpApA initiator and a labeled pppG or pppA terminator (SEQ ID NOs: 11 and 12).

Please amend paragraph [00146] at page 54 as follows:

[00146] FIG 23. Detection of telomerase activity with reiterative oligonucleotide synthesis. Reiterative oligonucleotide synthesis with DNA polymerases can also be used for signal generation, however, the product oligonucleotides need not be released, but may be joined tandemly in the product. As an example, telomerase activity can be detected by immobilizing a telomerase-specific probe to a solid matrix to capture cellular telomerase, which carries its own RNA template for DNA synthesis. For example, with human telomerase, the RNA template on the enzyme encodes the DNA sequence GGGTTA. The capture probe may contain the sequence GGGTTA, which will be added reiteratively to the end of the telomerase capture probe, if telomerase is present in the sample. Signal generation can be achieved in several ways, one of which involves including one or more reporter tagged dNTPs in the synthesis reaction to produce a product that has multiple R_1 groups attached along the backbone of the DNA product. For detection, this product can then be hybridized to a complementary probe containing nucleotides with a second R group (R_2) attached that will hybridize to the R_1 labeled product. This will bring the R_1 and R_2 groups together for signal generation via FRET from between R_1 and R_2, or via other methods. Alternatively, telomerase may incorporate 2 labeled nucleotides in the

product DNA and look for energy transfer between the 2 labeled nucleotides in the single strand of DNA (SEQ ID NOs: 13, 14, 15 and 16).

Please amend paragraph [0102] at page 55 as follows:

[0102] FIG. 27. Portion of the contig sequence of the CDKN2A gene. The sequence represents a small portion of the contig starting at 856630 nucleotides from the start of the contig sequence. The sequence represents a CpG island. Contig number: NT_008410.4 (SEQ ID NO: 17).

Please amend paragraph [0105] at pages 56-57 as follows:

[0105] FIG.30 shows template sequences for the abortive transcription reactions shown in FIGS. 31-34. FIG. 30a: Poly[dG-dC] is a synthetic deoxyribonucleotide polymer of repeating dCpdG. Individual strands contain variable numbers of dinucleotide repeats (SEQ ID NO: 18). FIG 30b: Bubble complex 1 was made by annealing synthetic, partially complementary template and non-template strands. The vertical offset of the non-template strand represents the single-stranded, bubble portion of the molecule. The coordinate system is based on the downstream edge of the bubble. The unpaired bases next to the double-stranded segment are at position +1. Positions to the left (upstream) of position +1 are given negative numbers starting with –1. The coordinate system is used to indicate the position of the 3' ends of the ribonucleotide initiators. The 3' end of initiator AA is aligned at +1 and the 3' end of initiator AU is aligned at +2. The transcription reaction proceeds from left to right from 3' end of the initiator, according to theory (SEQ ID NOs: 19 and 20). FIG. 30c represents the template strand without the complementary non-template strand. The sequence is shown in the 3' to 5' orientation (SEQ ID NO: 21).

Please amend paragraph [0397] at page 161 as follows:

[0397] ATATACTGGGTCTACAAGGTTTAAGTCAACCAGGGATTGAAATATAA CTTTTAAACAGAGCTGG (SEQ ID NO: 1). The DNA sample is incubated with the capture probe to allow hybridization. A representative hybridization protocol is as follows: (1) prehybridize with 2.5X SSC, 5X Denhardts at room temperature for 30 minutes; (2) hybridize with 2.5X SSC, 5X Denhardts, 30% formamide at room temperature for 2 hours; (3) wash twice with 1X SSC at 42°C for 10 minutes, maintaining 42°C; and (4) wash three times with 0.1X SSC at 42°C for 10 minutes, maintaining 42°C.